Fluorescence Sensing of Plant Foliage

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Abstract

Vegetation fluorescence spectra taken of different leaf conditions have robust broadband features, from which useful diagnostics of leaf type (grass, broadleaf, and needle leaf), leaf turgidity, and leaf color gradients. The objective was to determine the location of these strong emission features and the associated excitation and emission bands.

Introduction

A fluorescence spectrum is a sequence of adjoining spectral bands for which a material's emission photons are measured for any narrow excitation band. The assemblage of the emission spectra from many excitation bands forms a three-dimensional excitation-emission matrix (EEM) or the excitation-emission spectrum (EES). The spectra are taken by scanning the emission bands from 350-850nm at 5nm intervals, and stepping the associated excitation bands from 250-750nm at 10nm intervals. The initial emission band wavelength is 20nm longer than the excitation band to avoid the Rayleigh peak. The three dimensional (3-D) EES plot is formed by the compilation of 3388 Ex-Em band combinations; where excitation (x), emission (y), and intensity (z) form the axes of the 3-D array.

The EES were taken of leaf samples from grass, broadleaf deciduous, broadleaf evergreen, and needle leaf evergreen plants. The samples have different pigmentation or moisture contents. Spectral analysis found the emission maxima of these plant materials were most often associated two excitation bands, Ex 400nm and Ex 470nm, and five emission bands or regions, Em 470-495nm, Em 515nm, Em 520nm, Em 565nm, and Em 680-690nm (Figure 2). The dynamic range of emissions in the EES required a method for separating the primary emission centers from the background emissions within the EES. The emission intensity filter, used in the spectral analysis, was the sum of the mean and the standard deviation (SD) of all emission intensities greater than zero. This intensity metric was the filter that differentiated the strong emission centers within the EES, from which it was calculated

Green-Colored, Turgid Leaves.

Turgid, green-color leaves from grasses and broadleaf plants, i.e. pepper, poinsettia, bush bean, soybean, and sweet gum have two broadband emissions or centroids. The primary region is an ellipsoid shaped centroid bounded by the Em 680-750nm, and the Ex 400-650nm bands. This centroid has a bimodal cross-section with emission peaks at Em 685nm and Em 730nm and two excitation bands, Ex 400nm and Ex 470nm. The red and far-red emissions, Em 685nm and EM 730nm, respectively, are associated with photo system-I and -II fluorescence.

Environmental factors affecting these photo-systems should affect the shape and amplitude of this centroid (Figure 1).

A second emission centroid and one of lower amplitude and smaller spatial dimensionality, is located in the UV-blue region. The Em 380nm-450nm and the Ex 350-410nm bands bound the ellipsoid shaped centroid. These emissions are associated with cellulose, cellulosic materials, and the yellow-

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pigments. This is a spectral region of chlorophyll absorption. Factors reducing leaf chlorophyll will affect the leaf emission intensities, which can vary by plant species (Figure 3).

Turgid, Colored-Leaves.

Visual color can be indicative of the plant's physiological condition and an indicator of plant vigor and health, and nutrient deficiencies or excesses. Fluorescence features in the red-far red have been used for pre-visual plant health/stress assessments, but these are based upon a few excitation-emission band combinations. The fluorescence spectra taken of senescing leaves from healthy soybean and sweet gum plants provide data for understanding the broader context of the leaf color-fluorescence spectral relationships.

Soybean senescence is along the green-, yellow-, and brown-color leaf sequence. The red-far red emissions of these leaves decrease as leaf greenness decreases, whereas and the UV-blue-green emissions increase along this color gradient. The leaf spectra are inter-twined and are spectrally confused in the Em 660-725nm region for many excitation bands. The green-yellow- and yellow-colored leaf emission spectra overlap in the Ex 350-380nm region but otherwise the yellow-colored leaf emissions are distinct over this region (Figure 4a).

Sweet gum leaf senesce is either along a green- to yellow-colored leaf pathway or through a green- to red-colored leaf pathway. The leaf color is highly variable on the same tree depending upon the solar illumination and tree canopy geometry. Generally emission intensities vary by leaf color, yellow>green>red>purple-colored leaves. The yellow-colored leaves have high red-far red emissions and no strong emissions in the UV-blue region. The red-colored leaves are an apparent transition phase leading to the purple-colored leaves. These leaves have chlorophyll intermix with the xanthophylls and anthanocyanin pigments on the leaf surface layer. The purple-colored leaf has xanthophylls and anthanocyanin pigments on the upper leaf surface and chlorophylls on the bottom leaf surface. The red and purple colored leaves have high red-far red emissions although their emissions are less than the green and yellow-colored leaves (Figure 4b).

Air-Dried Leaf Materials

The study of plant-water stress involves multiple intersecting factors, which change the leaf and the canopy features. The plant drought avoidance mechanisms can include reduced leaf cover, leaf area, and leaf biomass within the plant canopy. Initial drought induced stress can cause the leaves to be reoriented to reduced solar loading. In some species, the leaves roll-up or curl and in other species the leaves can wilt and droop, even though they remain attached to the plant. In severe drought stress, the leaf senesce is associated with a change of leaf color, either yellow-green or yellow color. These leaves can still have high water contents. Eventual leaf abscission can occur acropetally toward the younger leaves and the shoot apical meristem.

The EES of excised green-colored poinsettia leaves show the effect of water loss through desiccation, beginning with a turgid-green-colored leaf and ending with an air-dry, green-color leaf, which was then oven-dried. During the drying process, the red-far red emission centroid decreased in size and amplitude and the blue-green centroid enlarged (Figure 5a). The air-dried soybean leaves of different colors show this same transition from broad based emissions in the red-far red centroid to the blue-green region (Figure 5b).

Tan- and brown-colored, air-dry leaf samples have a significant emission centroid in the blue-green region, bounded by Em 400-650nm and Ex 340-530nm. These emission spectra are much brighter than those of the turgid, green-colored leaves. Although the centroids are similar spatially, their intensities vary by plant material. The glumes and newly senesced leaf samples have very high emissions and their centroids are broad based. Tan-colored soybean leaves have lower emissions compared with the senesced grass leaves. Weathered and aged plant materials have low emission intensities compared with the newly senesced materials, Figure 6.

The excised leaves subjected to desiccation are spectrally different compared with leaves from water stressed plant. The excised leaves undergo internal change but constituents are fixed in place, i.e., the chlorophylls, and materials are not translocated from the leaf. Upon air-drying the excised leaves retained much of their green color. The leaves stressed on the plant can undergo various physiological changes whereby leaf constituents, i.e., chlorophyll, are broken down and translocated from the leaf, before leaf abscission.

Application to Remotely Sensed Fluorescence Imagery

Leaf biomass, leaf area, and leaf cover are highly correlated, hence there are good reasons for expecting plant canopy emission differences if plant stresses alter the composition of the vegetation/soil mosaic. Typically the soils have low emission intensities, which are related to the fluorescence quenching elements and materials. Reduced canopy cover and increased soil would lower the emission intensities of the vegetation-soil mosaic. The presence of organic matter and leaf debris backgrounds or the yellow-green and yellow-colored leaves should enhance the blue-green fluorescence but the red-far red emissions would be less than the emission from the full green-color leaf canopy. The aging and decomposition of leaf and stem debris would reduce the emission intensities. Conversely, the red-far red emissions from the vegetation-soil mosaic would increase directly with vigorous plant growth and leaf development and maturation.

Conclusions

1. Two excitation bands, Ex400nm and Ex470nm, were commonly associated with the red-far red emission of green leaves. Three other excitation bands, Ex400nm, Ex450nm, and Ex370nm, were associated with the UV-blue emissions of tan- and yellow-colored leaves.

2. Different colored leaves and leaf water states have diagnostic emissions centers.

3. The vegetation emissions centroids are broadband features in the red-far red and UV-blue spectral regions, where the emission intensities can vary by leaf color, turgidity, and leaf type.

4. Remote sensing technologies can be used for change detection and for assessments of plant vigor. However, remotely differentiating and determining the probable causative factors from those associated with natural plant growth phenomena can present significant difficulties.

Figure 1a. Excitation-Emission Spectrum of Green-color Bean Leaf

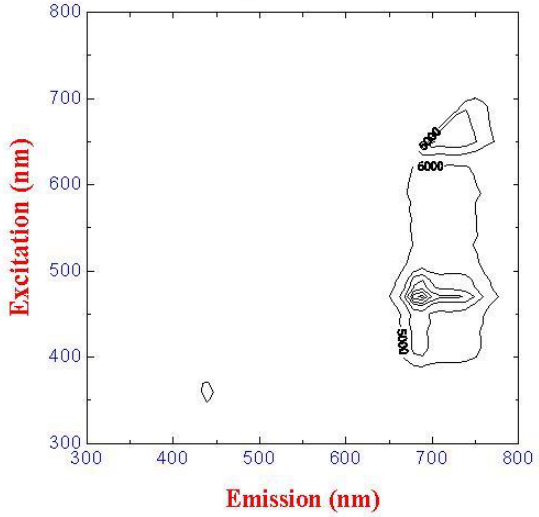

Figure 1b. Excitation-Emission Spectrum of Green-color Bean Leaf

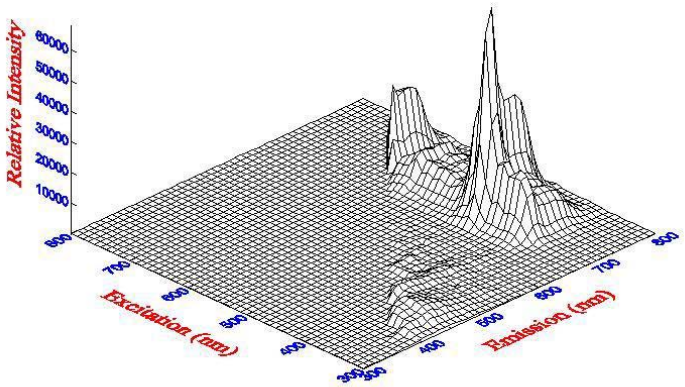

Figure 2a. Leaf and Stem Peak Excitation Bands

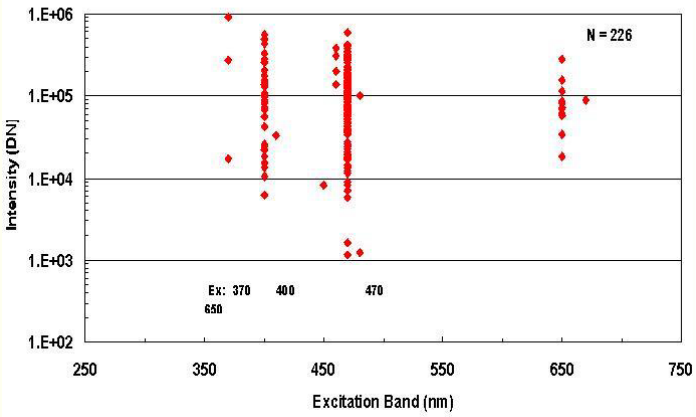

Figure 2b. Leaf and Stem Peak Emission Bands.

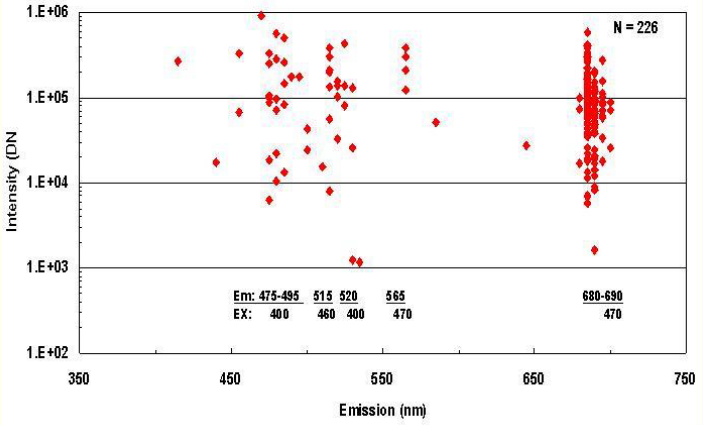

Figure 3a. Turgid, Green-Color Grass Leaf Samples

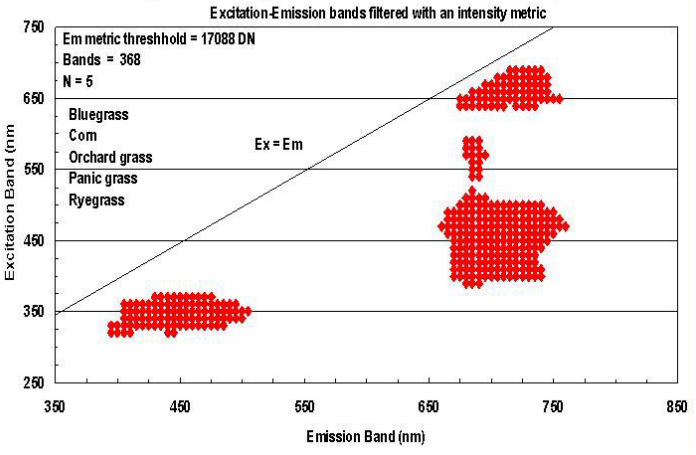

Figure 3b. Turgid, Green-colored Bean Leaves

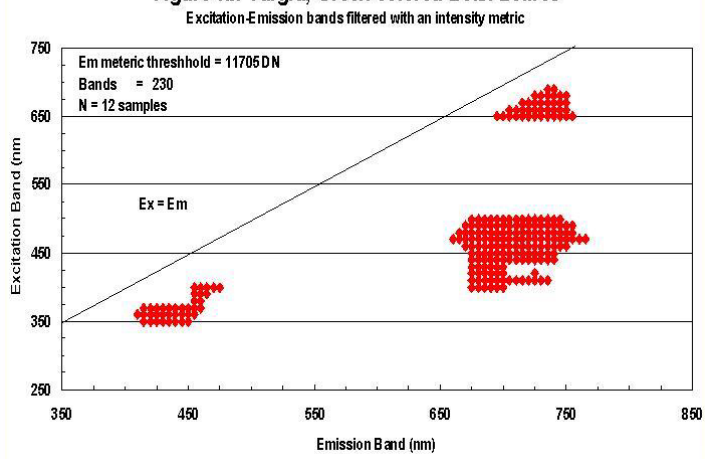

Figure 4a. Colored, Turgid Soybean Leaf Samples

Excitation-Emission bands filtered with an intensity metric

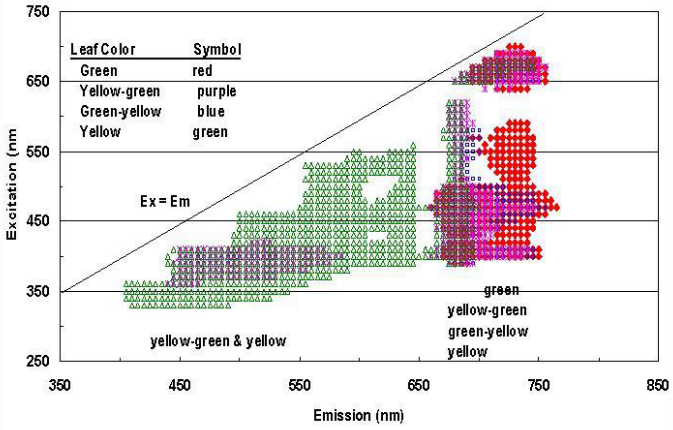

Figure 4b. Colored Turgid Sweet Gum Leaf Samples

Emission-Excitation bands filtered with an intensity metric

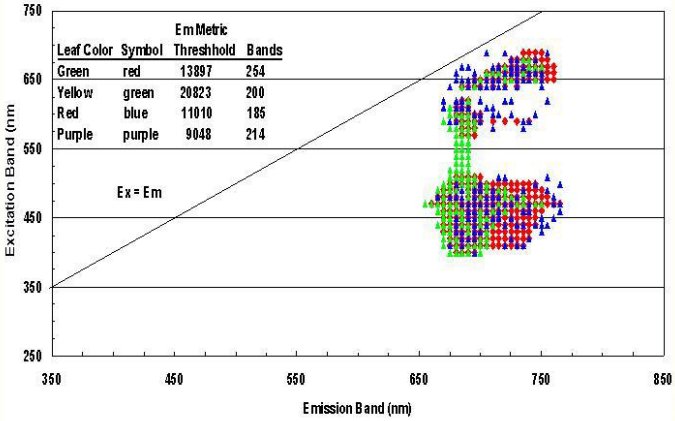

Figure 5a. Green-Colored Poinsettia Leaf Samples

Excitation-Emission bands filtered with an intensity metric

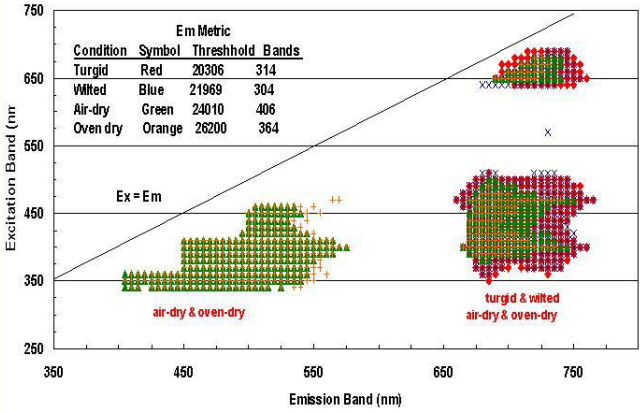

Figure 5b. Turgid and Air-dry Soybean Leaf Samples

Excitation-Emission bands filtered with an intensity metric

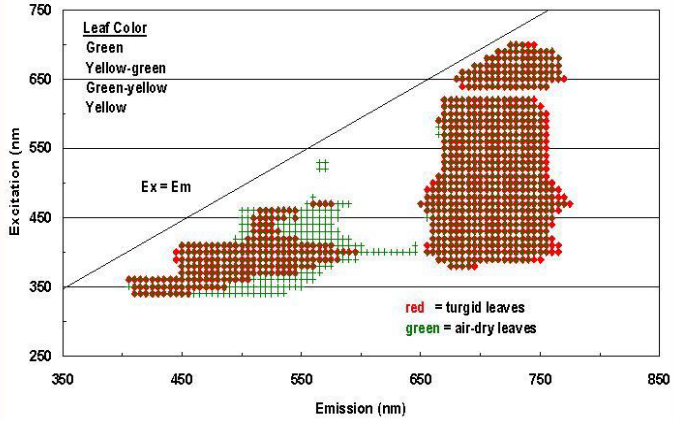

Figure 6a. Air-dry, Tan-color Wheat Leaf and Culm Samples

Excitation-Emission bands filtered with an intensity metric

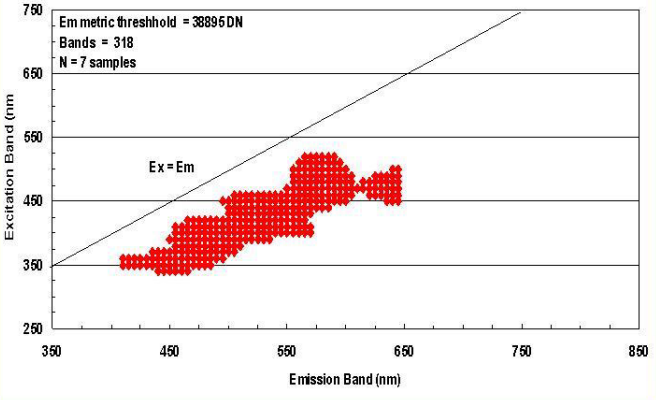

Figure 6b. Air-dry Corn Leaf Samples, New & Old

Excitation-Emission bands filtered with an intensity metric.